BIOASSAY, CARCINOGENESIS and TISSUE CULTURE March 1, 1962

COMMITTEE:
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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street
New York 17, New York

#343
(Compare #295
denied 2/10/61)

APPLICATION FOR RESEARCH GRANT

Date: February 13, 1962

1. Name of Investigator: MARTIN S. PROTZEL, B.S., D.D.S.

2. Title: Chief, Department of Oral Pathology

3. Institution & Address: Martland Medical Center
65 Bergen Street
Newark, New Jersey

4. Project or Subject: "A COMPARATIVE STUDY OF THE EFFECTS OF 3-METHYLCHOLANTHRENE AND CIGARETTE SMOKE CONDENSATE APPLIED TO THE ORAL TISSUES OF SWISS MICE (ICR) CONDITIONED WITH ALCOHOL AND/OR CARBON TETRACHLORIDE".

5. Detailed Plan of Procedure: Randombred Swiss Mice (ICR) will be employed as the experimental animal. One group of animals (A) will be painted with a 0.3% solution of 3-methylcholanthrene in acetone on the mucosal surfaces of the lip and cheek. A second group of animals (B) will be painted with a 50% solution of cigarette smoke condensate in acetone at the same sites as animals in group "A". Animals in the control group (C) will be painted with an isotonic saline solution at the same sites as animals in groups "A" and "B". All groups of animals will be painted four times weekly; a cotton applicator will serve as the instrument for swabbing the oral tissues. Animal groups "A", "B" and "C" will be subdivided into four sub-groups. Animals in Groups "A"-1, "B"-1, "C"-1 will have a 15% ethyl alcohol solution in water for drinking, ad libitum and will be given a subcutaneous injection once every week of 0.06 cc of a 50% solution of carbon tetrachloride in mineral oil. Animals in groups "A"-2, "B"-2, "C"-2 will have the alcohol for drinking however, the carbon tetrachloride will not be injected. Animals in groups "A"-3, "B"-3, "C"-3 will have water for drinking, ad libitum, however, they will be injected with carbon tetrachloride. Animals in groups "A"-4, "B"-4, "C"-4, will have water for drinking, ad libitum, carbon tetrachloride will not be injected. Ninety-six animals will comprise each group with twenty-four in each sub-group for a total number of two hundred and eighty-eight. Each sub-group will have twelve males and twelve females. Animals will be housed in stainless steel cages, six to a cage. A standard laboratory chow for mice will be provided. Cages will be cleaned and sterilized once a week. Animals will be observed daily. Initial and progressive changes noted clinically will be recorded and photographed. When a malignant process is observed, the animal will be sacrificed by the exsanguination method. Blood will be obtained from the right ventricle of the heart and will be collected in sterile tubes, then sent to the Biochemistry Department for electrophoretic

studies of serum proteins (total protein, Albumin, alpha 1, alpha 2, Beta and Gamma globulins). Individual electrophoretic patterns will be traced on graph paper and evaluated. Complete autopsies will be performed on each animal, special attention will be given to the analysis of the oral tissues, liver and cervical lymph nodes. All organs, however, will be carefully studied grossly and microscopically. The livers of all animals will be stained with Sudan IV as well as routine H.and E. for microscopic analysis.

6. Budget Plan:

a.	Salaries	$14,000.00
b.	Expendable Supplies	1,827.00
c.	Permanent Equipment	3,005.00
d.	Overhead (15% of a, b, e)	2,449.05
e.	Other	500.00
	TOTAL	$21,781.05

7. Anticipated Duration of Work: ONE YEAR

8. Facilities and Staff Available:

There are four animal rooms available adjacent to one another on a special floor in the hospital. Each room is approximately 25' x 15' with finished walls and vinyl floors. Modern sinks and cabinets are present in each room. One room contains a refrigerator and two steam sterilizers. Mobile Mayo surgical stands and castle operating room lights are available as well as a surgical cabinet with suitable instruments. Two rooms are being equipped with air conditioners. The pathology, hematology and biochemistry laboratories are well equipped and staffed to render the service and material for this project. The animal cages (30) and cage stands (2) however, are constructed of galvanized iron and are inadequate both in number and composition for this project.

The members of the staff available for this project include Dr. Edwin H. Albano, Dr. Thomas A. Santoro, of the Department of Pathology of the Martland Medical Center. Laboratory assistants in all of the departments of Pathology are also available.

9. Additional Requirements:

Stainless steel cages for mice, with racks
Assistant for the animal caretaker
Food Containers
Air conditioners (2) one-ton units

10. Additional information (including relation of work to other projects and other sources of supply)

During the past 20 years, I have examined scores of patients with oral cancer. Consistently, these patients yielded histories of drinking alcoholic beverages over a long period of time, and admitted to the excessive use of tobacco. Clinically, these patients also exhibited concomittant evidence of dietary deficiencies. It is my opinion that individuals who are constant drinkers of alcoholic beverages and have concommittant dietary deficiencies develop liver damage. Liver damage with functional imbalance over a period of time renders

the oral mucosal tissues more susceptible to the action of chemical carcinogens. It is possible, therefore, that liver imbalance which precedes the development of oral cancer is an important cocarcinogenic factor. Thus, over a long period of time, a weak carcinogen acting constantly on a susceptible oral mucosa could induce cancer. Statistical studies of Trieger, Taylor and Weisberger and R. C. Vincent of Roswell Park Memorial Institute confirm these observations. In a recent article in the Journal of the American Medical Association, Herbut, Tsaltus and Kraemer suggest the possibility of a tumor inhibitory principle present in the liver which when deficient, either general or local, could be responsible for the development of cancer. To determine whether liver damage is an important cocarcinogenic factor in the development of oral cancer, a preliminary animal experiment was performed at the Martland Medical Center Laboratory. This study was completed recently and is being prepared for publication. For this study, thirty randombred Swiss Albino mice were employed. Liver damage in the form of fatty infiltration was induced by the weekly injection of a 0.06 cc solution of carbon tetrachloride in mineral oil and/or the use of a 15% solution of ethyl alcohol for drinking. The chemical carcinogen was a 3-methylcholanthrene which was employed as a 0.3% solution in acetone and applied to the oral mucosal surfaces of the lip and cheek of the animals four times weekly. Animals painted with the carcinogen and receiving carbon tetrachloride and alcohol developed tumors eight weeks sooner than the animals which were merely painted with the carcinogen. Malignant changes from initial papilloma to frank carcinoma developed more rapidly in the former group of animals than in the latter. Histologically, the malignant tumors were squamous cell carcinoma varying from Grade I to Grade III. Employing routine H. and E. and special Fat Stain, Sudan IV, the livers of these animals exhibited fatty infiltration. Electropheretic studies of the proteins of these animals disclosed abnormalities of albumin, albumin-globulin ratio and alpha, beta and gamma globulin. These results indicated liver imbalance. Although not conclusive, the sum total of these results indicated that when liver imbalance exists in the experimental animal, the oral mucosal tissues are more susceptible to the action of a chemical carcinogen. We realize that the use of thirty animals for this study represents too small a number for critical or conclusive evaluation. It is possible, however, that results obtained employing a large number of animals (288) subjected to the same experimental procedures performed in the preliminary study would prove to be more conclusive.

Signature MARTIN S. PROTZEL, B.S., D.D.S.
Director of Project

G. O. ROTUNDA
Business Officer of the Institution

REFERENCE TO PUBLISHED ARTICLES APPLICABLE TO THIS EXPERIMENT

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